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Molecular insights into the force-from-lipids gating of mechanosensitive channels

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It is well-established that mechanosensitive (MS) ion channels differentially respond to membrane tension, bilayer thinning, and curvature. The thesis that the lipid bilayer acted as the terminal transducer of force directly to the channel became known as the force-from-lipids gating paradigm (also less frequently referred to as the 'bilayer model'). This principle allows cells to detect and respond to mechanical forces in their environment, which is important for various physiological processes, including blood pressure regulation, touch sensation, and many others. Our understanding of how mechanical force drives MS channel gating has been greatly enhanced by new insights into the molecular interactions between the lipid bilayer and channel proteins. In this short review, we revisit the role of the force-from-lipids principle within the current understanding of MS channel gating and focus on its molecular underpinnings.

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Introduction

Since the very beginning of life, osmotic pressure has existed as a prevalent mechanical force. Examples of molecules that have facilitated sensing these forces include the bacterial mechanosensitive (MS) ion channels (MscL and MscS). These evolutionarily ancient channels are activated by membrane tension and serve as osmotic safety valves that protect bacteria from excessive turgor pressures [1]. Furthermore, water, as a key evolutionary factor can be directly involved in the hydration of ion channel pores and induce subsequent conformational changes [3–5]. In this context, we explore the molecular mechanisms that MS channels have evolved over time to decipher mechanical cues, with a primary emphasis on the force-from-lipids paradigm. Additionally, we delve into the application of these fundamental molecular principles to 'modern' mechanosensors, encompassing the detection of hearing, touch, proprioception, and other mechanosensory modalities.

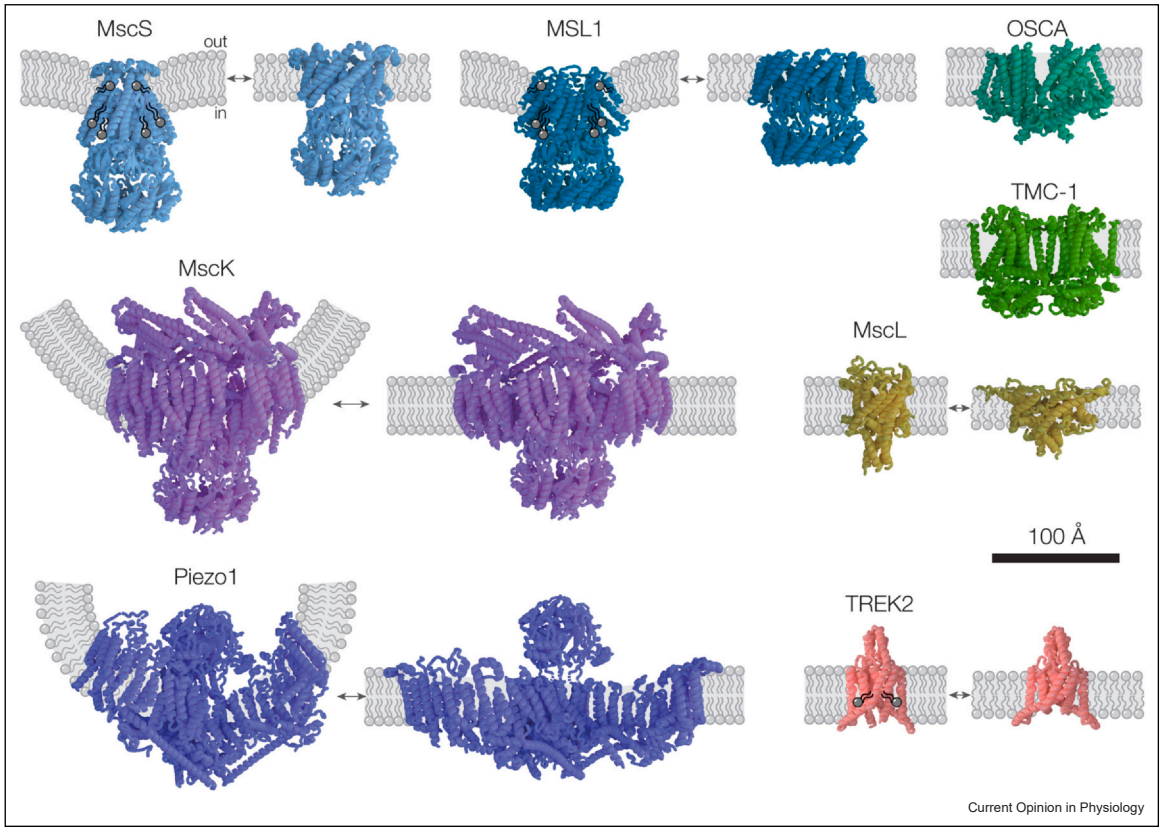
Structurally diverse mechanosensitive channel families

Among membrane mechanosensors, MS ion channels represent a class of primary transducers of mechanical force. These molecules have evolved to convert membrane deformation into electrical and/or biochemical signals. MscS- and MscL-like families of ion channels are found in prokaryotic cells of Bacteria and Archaea and are essential for osmoregulation [2,3]. The MscS-like (MSL) channel family also displays noticeable expression in plants, where they are expressed in a variety of cell types and tissues, including roots, leaves, and stems. They play a role in the response to mechanical stimuli, such as wind, gravity, and touch, and in the regulation of various processes, including growth, development, and osmotic stress responses [4–6].

Eukaryotic MS channels show great structural and functional diversity. They include TREK-1, TREK-2, and TRAAK, three members of the two-pore-domain (K_{2P}) weakly inward- rectifying K^+ channel family [7,8], ENaC, the epithelial amiloride-sensitive Na^+ channel, a member of the MEC/DEG (for 'mechanosensory abnormal/degenerins') family [9], the PIEZO channel family [10–12], and the OSCA/TMEM63 family of MS channels [13] (Figure 1).

In addition, transmembrane channel-like TMC proteins found in cells of the auditory system are thought to form the pore of the MS channel complex involved in hearing

Figure 1



Structural dynamics of MS channels based on the force-from-lipids principle. Structurally diverse representative members of MS channel families respond to bilayer forces such as tension, membrane thinning, and local curvature. Stretching and bending are two major ways to mechanically distort the lipid bilayer. Both deformations have been shown to affect gating of MS channels. (Note: Although the association between MscK opening and the generation of membrane curvature has not been explicitly demonstrated, new discoveries may eventually confirm or refute it.) MscS, MSL1, MscK, and MscL are members of bacterial-type MS channels found also in plants, archaea, and animal and human parasites, PIEZO1 is a member of the PIEZO channel family, OSCA channels form a large family of eukaryotic MS channels, and TREK-2 is a mechanosenitive member of the K2P ion channel family, whereas TMC1 is a TMC protein proposed to function as the mechanotransduction channel in hearing. Cartoons depict schematic representations of the membrane footprint for various structural conformations of different MS channels. These conformations have been reported with the following PDB IDs: MscS (6PWN https://doi.org/10.7554/eLife.50486, 8DDJ https://doi.org/10.7554/eLife.81445), MSL1 (6VXM, 6VXN https://doi.org/10.1038/s41467–020–17538–1), MscK (7UW5, 7UX1 https://doi.org/10.1038/s41467–022–34737–0), MscL (2OAR https://doi.org/10.1016/S1063–5823(06)58001–9, MD simulation https://doi.org/10.1038/ncomms11984, https://doi.org/10.1080/19336950.2016.1249077), OSCA (6MGW https://doi.org/10.7554/eLife.41845), TMC1 (7USX https://doi.org/10.1038/s41586–022–05314–8), Piezo1 (7WLT, 7WLU https://doi.org/10.1038/s41586–022–04574–8), and TREK-2 (4XDJ, 4BW5 https://doi.org/10.1126/science.1261512).

[14]. These molecules are part of the apparatus that enables sound waves to be converted into electrical signals by mechanical deformation of the tip links of the cochlear hair cells. Despite tremendous progress and structural determination of a TMC complex from *C. elegans* [15], much is still unknown about the force transduction mechanisms within the mammalian channel complex. The superfamily of transient receptor potential (for 'TRP') ion channels has also been linked to many MS processes [16,17]. Currently, stretch- activation of mammalian TRP channels is somewhat controversial. For example, the prevailing view has been that TRPV4 channels are stretch-activated and are involved in various physiological processes, including osmosensation, thermosensation, and mechanosensation. Early studies suggest that these channels could be activated by mechanical forces, such as cell stretching or shear stress, leading to calcium influx and subsequent cellular responses [22,23]. Recent research has challenged the direct stretch-activation of TRPV4 channels, and some studies have reported that the observed stretch-activation may be attributed to secondary effects, such as the release of endogenous ligands or indirect mechanotransduction pathways. If these channels are MS, then it is likely that they require auxiliary components to sense mechanical force. Moreover, compelling biochemical and structural data indicate that TRPV4 may sense mechanical force through direct interactions

with CSK auxiliary proteins, such as Rho GTPases [24–26]. However, additional functional data are necessary to elucidate the precise mechanism by which TRPV4 senses mechanical force in heterologous systems. This controversy is complicated by factors such as the variability in experimental methods, cell types, and expression systems used in different studies, thus contributing to the contrasting findings reported in the literature. Like TRPV4, stretch-activation of Yvc1/TRPY found in yeast is still being investigated. Some models suggest that mechanical forces directly influence the conformational changes of the Yvc1/TRPY channel, leading to its opening. Other studies, however, have suggested that mechanical stretch indirectly activates the channel through upstream signaling events involving various proteins and lipid components. While the understanding of Yvc1/TRPY channel activation is still evolving, current literature largely supports the involvement of Yvc1/TRPY in mechanosensation and its activation by mechanical stimuli, including membrane stretch [27,28]. Finally, only Drosophila TRPN (NOMPC) channels that play a role in gentle touch sensation and locomotion [18] have conclusively been shown to be primary mechanoreceptors activated by membrane stretch. Thus, the vast majority of mammalian TRP channels (with some notable exceptions) [19] do not seem to function as primary mechanosensors with their mechanosensitivity being a continual subject of debate [20,21].

Force-from-lipids principle for gating of mechanosensitive ion channels

Most diverse MS channel families have been shown to respond directly to membrane forces. The 'force-from-lipids' principle of gating MS channels by mechanical stimuli originated from patch-clamp studies of MscS and MscL, bacterial MS channels of small and large conductance, respectively [22]. The principle suggests that the force necessary to drive the channel from the closed to the open state is transmitted directly via the surrounding lipid bilayer (Figure 1). This principle arose from observations made from two separate experimental paradigms, both relying heavily on the improvements in the patch- clamp technique that occurred at the beginning of the 1980s [23]. The first was the observation that asymmetric incorporation of small amphipathic molecules into giant spheroplast membranes influenced the activation of bacterial MS channels [24]. A follow-up paper proposed an initial theoretical model of the action of amphipaths, including estimates of the amphipath partition into the lipid bilayer required for MS channel activation [36]. The second was the observation that when purified, MscL from *E. coli* could still be mechanically activated when reconstituted into liposomes, in the absence of other cellular components [25–27]. Since then, discussion and refinement of MS channel gating by the force-from-lipids paradigm have continued [40–44].

Ever-increasing interest in the force-form-lipids gating model has arisen from experiments showing that structurally and evolutionarily distinct eukaryotic MS channel families, in particular members of the K_{2P} channel family [28,29], PIEZO1 channels [30,31], and OSCA channels [13], also retained their mechanosensitivity in simplified systems in the absence of extraneous cellular components. While these reports do not preclude a role for the cytoskeleton or extracellular matrix in the gating modulation [32] and activation [33] of these channels in situ, it suggests that each channel is endowed with the structural requirements to respond to changes in membrane forces. Given that the bacterial channels (MscS, MscL) and the eukaryotic MS channels (OSCAs, TREK/TRAAK, and PIEZO1) are structurally diverse, this raises an important and relevant question of whether there are common biophysical or structural aspects of membrane-mediated channel activation. Or perhaps evolution has generated different 'structural answers' to a variety of 'mechanical questions', leading to unique MS channel gating mechanisms. Indeed, to this end, structural biology techniques in combination with functional studies have been used to generate MS channel-specific 'molecular interpretations' of the force-from-lipids gating model [34–36]. The remainder of this review looks specifically at these molecular interpretations of the force-from-lipids gating model and whether there are underlying conserved mechanisms between MS channel families.

Membrane tension and local curvature

Force-from-lipids can manifest in the form of membrane tension and/or local changes in membrane curvature. Membrane tension refers to the forces that act on the surface of a cell membrane such as those exerted due to internal turgor pressure [37,38] or by the cytoskeleton on the plasma membrane [39,40]. In addition to osmoregulation, membrane tension also plays a role in cell migration, division, and the development of tissues and organs, as it helps to ensure that cells are able to maintain their proper shape and position during these processes [41,42]. Notably, a recent study shows that MS TRAAK K^+ channel can be activated by lateral membrane tension decoupled from membrane curvature. This was shown through development of a bilayer tensiometer, allowing precise measurement of the membrane tension required to activate MS ion channels [60]. However, activation of TRAAK has also been observed upon unilateral insertion of the lysophospholipid LPC into a membrane bilayer, indicating possible activation of the channel by local membrane curvature [61].

Bending or curving the membrane can occur in response to changes in membrane tension or as a result of other factors, such as the presence of membrane proteins that can alter the shape of the membrane [43,44]. Importantly, membrane tension and curvature are interconnected and can affect each other on numerous levels [45–47]. Changes in membrane tension can result in changes in membrane curvature, and vice versa. Energetic calculations show that only local curvatures (on the order of ≤ 50 nm) induce sufficient pressure profile asymmetries (corresponding to tension of several pN/nm) to drive MS channel gating [67].

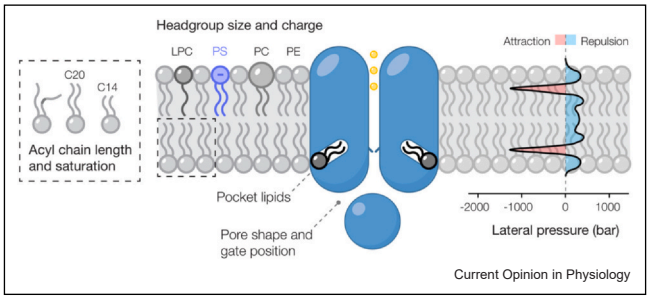
Physical forces within the membrane (e.g. membrane tension and bending) can not only drive gating transitions in MS channels (Figure 1), but can affect the organization of membrane proteins [48]. For example, high membrane tension may reduce the mobility of membrane proteins and cause them to become more densely packed within the membrane [49]. Moreover, membrane proteins can be classified based on their preference for different curvature states, such as positive, negative, or neutral curvature. Some proteins are more likely to partition to regions of the membrane with a particular curvature, while others may be more flexible and able to adapt to a range of curvature states [50,51]. Although mechanical force can affect any membrane protein depending on the force magnitude, mechanosensors such as MS channels are specialized membrane proteins, whose function is affected by physiologically relevant membrane tension and changes in local membrane curvature. Here, it is important to note that the final piece of the puzzle is reconciling the lower levels of membrane tension measured in native cells (~0.01–0.3 pN/nm) [52,53] to the somewhat higher ranges of tension associated with MS channel activation (> 0.5 pN/nm) [54]. A possible reason for which can be a lack of information regarding the exact force magnitude reaching MS channels in native cells.

As a prime example of how curvature can influence MS channel behavior, it has recently been demonstrated that local (nanoscale) membrane curvature affects the spatial distribution of PIEZO1 in the membrane [75]. PIEZO1 avoids localization at highly curved membrane protrusions such as filopodia, instead, it enriches in membrane invaginations. Importantly, here, the length scales of this curvature-dependent sorting of PIEZO1 argue against an enrichment in caveolae specifically. Interestingly, the channel density on filopodia increases upon channel activation, suggesting flattening of the channel upon opening. This result is consistent with recent structural studies where PIEZO1 is shown to adopt a potentially open flattened configuration when incorporated into small liposomes [76]. This flattening transition agrees with high-speed atomic force microscopy on individual PIEZO1 proteins [55]. Curvature-dependent sorting of PIEZO1 is also supported by another recent paper in native erythrocyte membranes where the PIEZO1 channel "exhibits the properties of a 'force-through-membrane (sic)' sensor of curvature and lateral tension" [56]. Owing to the energetic coupling between the curved shape of PIEZO1 and the curvature of the erythrocyte biconcave discs, they follow local and global changes in the erythrocyte membrane curvature and concentrate within the biconcave area. The influence of curvature on MS channel activation and localization is likely to be a key driver of their physiological function.

Mechanosensitive channels are differentially modulated by amphipaths

In addition to membrane stretch and local curvature, MS channels can also be activated by incorporation of amphipaths. This results from the intimate relationship between MS channels and membrane lipids, meaning that amphipathic compounds (e.g. chlorpromazine, local anesthetics, and lysolipids) inserted into one monolayer of the lipid bilayer can cause channel activation [57–59]. Whether this is caused by local membrane curvature or changes in transbilayer pressure profile without local curvature is still unknown (Figure 2). The influence of an amphipath depends on the structure of the MS channel and the leaflet in which the amphipath inserts. The effect of two amphipaths, Fluorouracil (5-FU), a chemotherapy agent [44], and the anesthetic trifluoroethanol [82], was tested on structurally distinct MscL, MscS, and TREK-1 MS channels. Both amphipaths affected the channels differently according to their size and shape as well as into which lipid monolayer they were incorporated into [44]. The finding of activation of MS channels by unilateral insertion of amphipathic compounds seems physiologically relevant, given that many physiologically active molecules have amphipathic properties, such as arachidonic acid that was shown to

Figure 2



Differential modulation of MS channels by different lipids. Changes in the lipid composition can change the global mechanical properties of the membrane, such as its stiffness, by altering the lateral pressure profile, intrinsic curvature, and thickness. Such changes include the degree of unsaturation, chain length, charge, shape, and thickness of the lipid molecule. Lipids can also differentially interact with the channel protein by occluding functionally important 'pockets' within the MS channel structure (structural lipids), thus tuning their function.

activate MS NMDA receptors and K_2P channels [83–85]. This regulation of membrane protein function through asymmetric partitioning of amphipathic molecules in the membrane may represent a general regulatory mechanism in various biological systems, extending beyond the channels mentioned. Nevertheless, the exact mechanism of how insertion of amphipaths transiently changes the bilayer properties such as pressure profile, thickness, and curvature and how this is coupled to MS channel modulation is still unclear.

Hydrophobic mismatch, lipid unsaturation, and mechanical properties of the membrane

In addition to bending rigidity, membrane thickness can affect MS channels through a lipid–protein interaction called 'hydrophobic mismatch'. To understand the hydrophobic surface match model, it is important to remember that stretching of the elastic bilayer leads to an increase of the area occupied by lipid molecules, which also results in a proportional decrease in bilayer thickness, given that the lipid bilayer behaves as an incompressible fluid [86]. In this model, the hydrophobic match between the bilayer thickness and the length of the hydrophobic surface of the membrane protein is important for the stability of different protein conformations as reported for gramicidin A and MscL channels. Whereas a thicker compared with a thinner bilayer resulted in conversion of gramicidin A from a stretch-activated to a stretch-inactivated channel [60], MscL required less energy for activation by membrane stretch when placed in a thinner bilayer compared with a thicker bilayer [58]. Similar effects have been shown for TREK-2 channels [61] as well. At present, it is unknown how membrane thickness affects PIEZO or OSCA channels.

MscS seems to be less sensitive to bilayer thickness changes than MscL [62]. For example, by reconstituting MscS into thin lipids (e.g. PC14:0 and PC12:0), Zhang et al (2021) show that despite the MscS lipid facing helices (TM1 and TM2) undergoing a large tilt, the channel pore remains closed [36]. However, MscS is not completely insensitive to thickness as patch- clamp electrophysiology does show a small shift in tension sensitivity in thinner lipids (18:0 vs. 18:1 vs. 16:1) [63]. It is important here to note that the degree of unsaturation has a strong effect on both membrane thickness and bending rigidity [64]. Moreover, a recent study shows that the channel pore can be expanded toward an open state if PC14:1 is used as the main lipid in nanodiscs [65]. This could involve membrane thickness driving the channel toward the open state (14:1 is thinner than 14:0) but would seem more likely to be due to an effect of lipid unsaturation on multiple bilayer properties (e.g. bending rigidity and thickness) as opposed to an effect of thickness alone. Consistent with this, we know that unsaturation affects not only MscS channel gating but also PIEZO1 channel gating [63,66,67]. In addition to the degree of unsaturation and chain length, also charge of the lipid molecules affects MS channel gating properties (Figure 2) [68,69]. The relative influence of each lipid property on gating is likely to be channel-dependent.

Lipid-filled pockets and structural lipids

To understand force-from-lipids gating at the molecular level, it is important to appreciate the discrete molecular structure of the lipid bilayer and embedded membrane proteins. To this end, recent advances in single-particle cryo-electron microscopy (Cryo-EM) and nanodisc reconstitution of MS channels have provided new insights about the positioning of the channels in their native bilayer environment in addition to the 'structural lipids' bound to MS channels [35,36,70]. Structural lipids refer to lipids that are carried over upon detergent solubilization and contribute to the overall structure or function of the channel protein. They also support the stability and integrity of the channel protein and affect its oligomerization and gating properties [71,72]. In the case of MscS (Figure 2), two types of lipid densities are explicitly observed to interact with the channel even in a detergent solution: a phospholipid that 'hooks' the top of each TM2–TM3 hairpin and likely plays a role in force sensing, and a bundle of acyl chains occluding the permeation path called 'pore lipids' [53] [100].

A recent cryo-EM study characterized the structural dynamics of the MscS MS channels by showing sequential transitions between different channel conformations in different membrane environments, including one that mimicked membrane tension [36]. According to the study, the complete conformational cycle of MscS consists of structural states transiting from the closed to the open state followed by a subconducting and ultimately an inactivated state. The structural dynamics of the channel is a consequence of removing lipid molecules from the grooves and pockets in the channel structure by membrane tension generated by addition of β-cyclodextrin to the lipid bilayer containing the channel. This approach using cyclodextrins to modify 'force-from-lipids' has also shown promise for other structurally unrelated MS channel proteins [73]. It will be exciting to see if cyclodextrins can facilitate the acquisition of novel force-dependent conformations of other MS channels. This is fully in accordance with work showing that hydrophobic pockets in MscS are likely to be differentially interdigitated with lipids during the gating cycle prompting the authors to originally propose the 'lipids-move-first' model as a molecular interpretation of force-from-lipids [74].

Using high-resolution cryo-EM structures, another recent study conclusively shows that delipidation (or removal of the structural lipids) causes channel opening [34]. After addition of detergent-solubilized lipids, the channel closes again. A possible mechanism underlying this process indicates that gating occurs through specific lipid–protein interactions, whereby membrane tension, such as the tension applied during patch-clamp recording, removes the lipids from the hydrophobic grooves (pockets). Such pockets and grooves may also be present in other MS channels. This lipid removal allows the channel protein to change its conformation to assume alternate states. Whether this transition also requires pulling or dragging of the protein structure via protein–lipid interactions is currently unknown [75]. There is additionally, an emerging scenario based on the energetics of the global bilayer morphology around the MS channel between the closed and open state. In the absence of membrane tension, the bilayer is deformed around the channel, such that it is bent, thinned, or thickened at places, depending on the MS channel structure [65]. When the bilayer is stretched, it is energetically more costly for the bilayer to adopt the same morphology or footprint as in the resting state, since it is more difficult to bend a material that is under tension. Using extensive MD simulations, Park et al. (2022) [65] suggest that membrane tension shifts the gating equilibrium toward the conductive state not because it disrupts the lipid–protein interactions, but because it requires a higher energetic cost for conformational changes of an MS channel in the membrane associated with the closed state [92]. Of course, such a scenario is highly dependent on the compositional heterogeneity of the membrane in which the channel is embedded, with lipid heterogeneity (e.g. length, charge, unsaturation etc.) reducing the energetic cost by 'molding' to the new shape of the channel. Despite this, similar propositions have been made, using continuum mechanics models for PIEZO channels based on the footprint imposed by closed state of the PIEZO1 channel [76,77], where membrane tension sensitizes PIEZO1 force sensitivity by reducing the close-to-open gating energy.

Unifying concepts for bilayer-mediated mechanotransduction

The force-from-lipids principle applies to all membrane proteins that undergo an area shape change upon application of mechanical stimuli to the cell membrane. In addition to MS channels, other membrane proteins, such as G-protein-coupled receptors [78,79] or the outer hair cell motor protein Prestin, are reported to respond to force [80,81]. Prestin moves electrical charges along the plasma membrane in response to changes in membrane tension and like MS channels, undergoes an area shape change [82,83] as, for example, reported for MscL and PIEZO1.

Studies primarily done on the bacterial MscL channel indicate that in MS channels gated according to the force-from-lipids principle, there are amphipathic helices running parallel to the membrane, which can convey membrane tension directly to the channel pore [75]. Although it remains to be conclusively shown for MS channels such as PIEZO or the TMC1/TMC2 [15] hearing mechanotransduction channel, it remains possible that several MS channels share a force-transducing structural blueprint built on amphipathic bilayer coupling helices [11,75].

Force-sharing mechanisms

Since the cell membrane not only consists of the lipid bilayer, but acts as a 'complex mechanosensor' as a whole [84], the extracellular and intracellular forces acting on the membrane have to be sensed and thus shared among all membrane components. The embedded mechanosensory membrane proteins are directly in contact with the lipid bilayer where they experience changes in the transbilayer pressure profile. However, membrane forces are heavily influenced by the local cytoskeletal environment. As a result, modification of the cytoskeleton can, even in the absence of direct interactions, regulate MS channel function with PIEZO1 being a prime example [32]. The previously proposed and discussed force-sharing models of MS channel gating [33,85–89] are consistent with this view. Thus, modulation of force-from-lipids-gated MS channels (and other membrane proteins) by cytoskeletal proteins and extracellular matrix proteins is likely to be ubiquitous and thus the mechanism by which this occurs requires careful dissection. For a full review on the role of cytoskeleton and extracellular matrix and their effect on PIEZO1 and PIEZO2 activity, we refer the readers to an excellent up-to-date review by [90].

Future directions

The force-from-lipids principle has played a significant role in our understanding of the biophysical principles underlying MS channel gating. Numerous studies investigating inherent mechanosensitivity of ion channels from bacteria to humans have clearly demonstrated that eukaryotic channels TREK-1, TRAAK, OSCA, and PIEZO1 are inherently MS similar to MscL or MscS (many of the studies are cited in [91]). Essential for establishing force-from-lipids as the general evolutionary-conserved physicochemical principle of MS channel gating has been liposome reconstitution of purified proteins. This reductionist approach has been used to investigate the inherent mechanosensitivity of ion channels free from other cellular components. What remains to be studied and documented is the inherent mechanosensitivity of primary membrane-embedded mechanosensors other than ion channels. Furthermore, while many channels can function in reduced systems

such as liposomes, it will be important to establish the preferred 'mechanism-of-force-transduction' in cellular environments, given that PIEZO1, for example, is gated by mechanical force in reduced systems, but its activity is also modulated by cytoskeletal proteins and putative attachments to the extracellular matrix. Thus, it will be necessary to establish the contribution of force from the bilayer, the cytoskeleton, and/or extracellular matrix to channel gating in situ. It will be equally as important to decipher whether in different cellular locations (e.g. cell junctions or cell matrix adhesions [92]) and under different force regimens if certain force transduction pathways to the channel gate predominate. This will enable us to determine the ubiquity and applicability of the force-from-lipids principle within complex mammalian systems.

With advances in structural approaches such as cryo-EM in liposomes, high-speed atomic force microscopy, and emerging approaches such as hydrogen/deuterium exchange mass spectrometry combined with atomistic computational methods and new tools such as cyclodextrins, to apply force, it will be possible to study the structural dynamics of MS channels with ever more detailed atomic snapshots. This will likely include our ability to track the sequence of lipid movements that are associated with channel gating transitions. Moreover, we should be able to determine the influence of membrane tension and local curvature on specific lipid–protein interactions and uncover the contributions of local and global bilayer properties in MS channel gating. Obtaining high-resolution structures of different MS channels in complex membrane mimics and in native cell membranes will surely inform us not only about possible channel conformations (e.g. closed, open, or inactivated) but also about the shape and arrangement of the bilayer in the channel vicinity. With methods such as cryo-EM, we may also be able to explore how other components such as extracellular or cytoskeletal elements and auxiliary subunits may contribute to MS channel gating and how this influences force-from-lipids gating paradigms. These are all exciting paths that will help us to understand the biophysics and physiology of MS channels at the molecular level.

Data Availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- • of special interest
- •• of outstanding interest

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